Art. XX.—*The Effect of Certain Chemical Substances on the Vitality of the Buds of Potato-Tubers, and their Disinfective Action on Potato Blight (Phytophthora infestans).*

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(With Plates LVII.–LX.).

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The treatment of potatoes, destined to serve as seed tubers, by immersion in aqueous solutions of various antiseptic reagents, as a preventive means against certain disease-producing organisms, chiefly parasitic fungi, is a practice which is gradually becoming more and more general.

The basis of the practice, at least in the hands of the majority of potato growers, is largely if not completely empirical. The criteria usually relied upon as to the effectiveness or success of this or that method of treatment against potato diseases and their dissemination, are essentially based on the crop-yield, and its comparative freedom from the disease which the treatment is tentatively designed to lessen or even eliminate.

Although broadly recognised as essentially antiseptic, or under certain conditions toxic in their action, little attention appears to have been devoted to the study of either the mode of action on, or the possible penetration of these reagents into, the tissue of the tuber: whether, for example, penetration of these chemical substances does or does not take place, and if the former alternative holds, whether penetration occurs exclusively through the skin, or through both buds and skin; or whether under certain conditions their action, though at first it may be effective and beneficial, becomes decidedly harmful, etc.

At the outset it is desirable to state that the original objective sought in this inquiry, namely, the evolution and application of a method of treatment which, while effectively annihilating the sprouting power of the treated tuber, and, in the case of blight-infected material, also that of the causal fungus (*Phytophthora infestans*, De Bary), yet would not greatly impair its storage, culinary and edible qualities, has to some extent undergone deviation.

The present brief paper is merely a resumé of certain experimental work performed, and the results which it has so far furnished. While the work recorded lacks that completeness in certain directions which must naturally accrue from a more detailed investigation, it has nevertheless been deemed advisable to record such definite stages as the inquiry has so far reached.

Briefly set out, the principal objects of the inquiry have been to ascertain the influence exercised under definite conditions of time, temperature and concentration, by certain antiseptic compounds of definite chemical composition, in aqueous solution, on blight-free or blight-infected tubers, particularly in regard to the annihilation of the buds, of the treated tuber, and in the case of blight-infected material, of the hibernating mycelium of the blight-causing fungus (*Phytophthora infestans*).

Material and Experimental Method.

Certain distinct types of Victorian tubers, specifically denoted in the text as thick or thin-skinned, and varying in their degree of maturity, served as experimental material. The earlier series of experiments refer exclusively to blight-free tubers, which were steeped in brine solutions of varying strengths. Following on these are experiments with similar blight-free material, steeped in aqueous sulphuric and boracic acid solutions. Succeeding these are the experimental results furnished by steeping material in other antiseptic steep solutions, and lastly are experimental results afforded by the treatment of blight-infected material in like solutions.

The method of experiment adopted has been to steep a definite number of tubers at the temperature of the laboratory (16° – 18° C) in aqueous solution of the different chemical

substances enumerated, for definite time periods; at the termination of each steeping experiment to wash the treated material, and then to divide it into separate lots, one of which was placed under ordinary dry storage conditions, a second planted in good garden soil, a third cooked immediately after treatment, and the residue of this last lot at a later interval. In the case of blight-infected material, these subsequent investigations were subjected to planting, cooking, storage, and also to "forcing" tests, in which the treated material was placed under optimal conditions of temperature and moisture for the rapid development of the hibernating blight-fungus.

Preparation of Steep Solutions.

Ordinary unpurified table salt (NaCl) was dissolved in tap water, and solutions of approximately 5 per cent., 10 per cent. brines, etc., were prepared. Aqueous solutions of sulphuric acid, boracic acid, phenol, formaldehyde, etc., of approximately the strengths indicated later on were similarly prepared, with ordinary commercial samples of each of these different reagents. The experiments were established by placing the tubers in tall glass cylinders, and pouring in a sufficient bulk of solution until the material was just completely covered. As no effective means of readily checking evaporation at the surface of the solution were available during the course of these experiments, and moreover as it is a difficulty which would present itself in actual practice in other than laboratory experiments, no attempt was made to control this factor.

Experimental Data. Brine Steeps.

(a) Blight-free peeled tubers, Victorian mature thick-skins. Entry of the solution into the storage tissue of tubers.

The lots of peeled tubers (5 each) were steeped in brines of 4.2 per cent., 9.32 per cent., and 17.91 per cent., strengths for five days (4.2 per cent.) and six days (9.32 per cent. and 17.91 per cent.). Specimens of the brine were removed at intervals, conveniently diluted to definite volumes, and estimations of their respective chloride contents carried out.

In the table beneath are given the amounts of chlorides per 100 c.c. of brine solution at 5 and 6 days.

Table 1.

Grams chlorides expressed as NaCl per 100 c.c. soln.				
At commencement	4.27	9.32	17.91
After 5 days ...	3.77	6.58	11.93
After 6 days ...	—	6.58	11.84
Percentage of salt withdrawn from 100 c.c. of solution, after 5 days	20.02	29.45	33.39
do. do. 6 days ...	—	33.24	33.89

As these quantitative data show, a considerable amount of salt passes by diffusion from the solution into the tissue of the peeled tuber. The titre of the external solution undergoes a gradual fall. This fall, probably rapid at first, gradually slows down, until at about five or six days a condition of equilibrium is attained. The principal feature of these experiments to be noted is the rapidity and order of magnitude of the process of diffusion which occurs in the case of the peeled tuber, a phenomenon which is, as we shall see, chiefly caused by the removal of the skin of the steeped object.

The above results do not give an entirely accurate measure of the passage of the solute from the solution into the tissue of the tuber, for during the course of these and similar experiments, evaporation of water occurs at the surface of the salt solution, and this tends to concentrate it. The values given above (and those in the succeeding tables) actually measure the resultant of two opposed processes, diffusion of salt from the solution into the tuber, and evaporation of water from the solution, processes which respectively tend to diminish and increase its concentration.

These data, however, sufficiently demonstrate (under the selected conditions of experiment), that common salt (NaCl) very freely diffuses into and is absorbed by the tissue of peeled tubers.

Direct estimations of the chloride content of the tissue of treated and untreated tubers definitely show that after treat-

ment considerable amounts of salt are absorbed, and moreover afford a more accurate measure of the order of magnitude of this process.

In these and similar determinations, the material, after desiccation at 105° C to 107° C, until its weight was approximately constant, was carefully and completely carbonised at a low red-heat, then repeatedly extracted with hot water; the aqueous extracts were filtered, and filtrate and washings, after cooling, were made up to a definite volume. Titrations of definite volumes of the filtrate were then carried out. The values calculated from these data are summarised in the table beneath.

TABLE 1A.

Percentages of moisture and chlorides calculated on air-dried tissue.

	Moisture	Chlorides (calculated as NaCl)	Approximate relative increase in salt content NaCl)
Peeled tubers (untreated) -	70.2	- 0.28	- 1
Peeled tubers after 6 days steeping in 10 per cent. brine	66.9	- 2.29	- 10
Peeled tubers after 6 days steeping in 20 per cent. brine	65.86	- 4.96	- 20

The tubers after treatment were found to have completely lost their originally solid texture, and to possess a rubber-like elasticity, a condition induced by the extensive plasmolysis of the more peripherally situated portions of the storage tissue.

It is of interest to note the occurrence, at a comparatively early stage in these steeping experiments, and notably in the weakest concentration of brine (5 per cent.), of marked bacterial development and activity. Thus in this instance the onset and rapid progress of the phenomenon even under the temperature conditions stated, render it necessary to terminate this experiment on the fifth day. In every instance bacterial growth was chiefly confined to the external solution, and the motile types present were those usually associated with starch degradation.

It is probable that extraction of certain proteins and their derivatives occurs more rapidly with a 5 per cent. brine than with the higher concentrations, although the possible inhibitive effect of these higher concentrations on bacterial activity is not excluded. The point is one for future investigation, and requires controlling by Kjeldahl nitrogen estimations, and the institution of "salting out" experiments. The fact remains that toward the third to sixth day the steep-solution becomes a veritable culture medium.

These suggestions regarding the manner in which brine functions are supported by the fact that in parallel experiments in which like material was steeped in plain water, bacterial development was not so advanced, as shown by both microscopical and macroscopical observation.

Blight-free Unpeeled Tubers.

The experimental results so far related indicate that when peeled tubers are steeped in brine solutions, salt penetrates rapidly and freely into the tissue, and plasmolysis and ultimately death of the cellular units take place.

It remained to endeavour to ascertain whether entry of salt takes place so freely when intact, unpeeled tubers are steeped in brine solutions.

In the table hereunder and those which follow, the summarised experimental data are typical of and selected from a mass of similar essays, in which three different types of tubers—

(1) mature thick-skinned Victorian tubers,

(2) less mature thin-skinned Victorian tubers,

(3) less mature thin-skinned Victorian tubers,

served as the experimental material.

At the outset it may be stated that non-removal of the skin of the tuber prior to steeping, effectively retards the entry of the solute. This fact is well shown by the results of the following typical experiment.

Experiment:—Two lots (5 each) mature thick-skinned Victorian tubers, steeped in brines (a) and (b) for ten days. First determinations of chloride content of brines made after three days' steeping and subsequently after 4, 8 and 10 days.

Table 2.

Grams chlorides (calculated as NaCl) per 100 c.c. of solution.

At commencement	...	(a) -	(b)
After 3 days' steeping ...	...	8.35 -	9.21
After 4 days' steeping .	...	8.41 -	9.27
After 8 days' steeping ..	...	8.50 -	9.30
After 10 days' steeping	...	8.47 -	9.24

Estimations of the moisture and chloride content made on this material yielded the following data:—

Table 2a.

Percentage of moisture and chlorides calculated on air-dried tissue.

	Moisture		Chlorides (calculated asNaCl)		Approximate relative increase in salt content
Intact tubers (untreated)	70.02	-	0.28	-	1
Do. after 10 days (a)	72.3	-	0.69	-	2
Steeping ... (b)	73.4	-	0.66	-	2

Additional experiments were then instituted, in order to follow more closely the behaviour of intact tubers, differing in type and degree of maturity, on steeping in brine solutions of different concentration, to accumulate data similar to those already amassed, and to extend the investigations along lines which might have certain practical applications.

Experiment:—Two lots (5 each) mature thick-skinned Victorian tubers, separately steeped in 10 per cent. and 15 per cent. brines for eight days.

Table 3.

Grams chlorides calculated as NaCl per 100 c.c. of solution.

	10 per cent.		15 per cent.
At commencement	9.65	-	14.53
After two days	9.71	-	14.43
After five days	9.83	-	14.46
After eight days	9.69	-	14.19

TABLE 3A.

Percentages of moisture and chlorides calculated on air-dried tissue.

	Moisture	Chlorides (calculated as NaCl)	Approximate relative increase in salt content
Intact tubers (untreated) ...	69.98	0.28	1
Intact tubers after 10 days' steeping, 10 per cent. ...	—	0.25	1
Intact tubers after 10 days' steeping, 15 per cent. ...	—	0.54	2

Experiment:—Two lots (5 each) less mature thin-skinned South Australian tubers, steeped in 15 per cent. brine for 11 days.

TABLE 4.

Grams chlorides calculated as NaCl per 100 c.c. of solution.

	(a)	(b)
At commencement	14.52	15.05
After 11 days' steep ...	14.22	15.17

Experiment:—Two lots (5 each) less mature thin-skinned Victorian tubers, separately steeped in 10 per cent. and 15 per cent. brines.

TABLE 4A.

	10 per cent.	15 per cent.
At commencement	9.69	14.19
After 8 days' steep	9.48	13.86

TABLE 4B.

Percentages of moisture and chlorides calculated on air-dried tissue.

	Moisture	Chlorides
South Australian tubers ...	70.67	0.66
Victorian tubers	70.60	0.89

These results justify the statement that, in contradistinction to peeled tubers, with unpeeled tubers, after long steeping in 10 per cent. or 15 per cent. brines, the amount of salt which diffuses from solution into the tuber is of a relatively small order of magnitude.

Since the behaviour of the unpeeled differs so distinctly from that of the peeled tuber, in regard to the entry of salt into its tissues, it is difficult to avoid the conclusion that the intact skin presents a barrier to the ready entry of the dissolved substance. As it is difficult to devise any satisfactory colorimetric method for demonstrating the presence of the small quantities of the chloride in the tissue, and of thus localising its points of entry, the further discussion of this aspect of the question may well be deferred until certain crucial experiments with tubers steeped in sulphuric acid are described and considered.

The brines in these experiments remained perfectly clear, and slight bacterial development only took place, demonstrating that practically little, if any, nutrient substances were extracted from the steeped objects.

Storage and cooking tests were most unsatisfactory. The stored tubers rapidly softened, lost weight, discoloured and finally decayed.

Specimens from each of the series were planted in good garden soil, and there remained for eight weeks. In every instance the results were negative, conclusively showing that the vitality of the dormant buds of the treated had been destroyed by the treatment.

Specimens of these brine-steeped peeled and unpeeled tubers, immediately after treatment, were cut in halves, and the cut surfaces freely exposed to the air. After 24 hours' exposure, the superficial tissue to a depth of several millimetres invariably acquired a greyish-black to black colour.

That this phenomenon is enzymatic in its nature is shown to be highly probable by the following experiments and observations.

Untreated tubers (A) and brine-steeped tubers (B) were bisected and exposed for 24 hours. Both series exhibited the progressive darkening of their exposed sectioned surfaces. After an hour's exposure a pinkish coloration commenced at the margin, later a liver-coloured tint, and still later a greyish black, and finally after the lapse of 24 hours a black coloration, the intensity and rapidity of the changes being more marked in series B.

The phenomenon is superficial, and its progress is governed by gradual desiccation of the surface tissue, and also probably by comparative lack of free oxygen in the subjacent tissue.

The superficial layers to a depth of a few millimetres were removed, and thin slices of the subjacent tissue in each series were prepared. These were divided into two lots, one of which was placed in water and heated nearly to boiling point and maintained at this temperature for some minutes. The boiled and unboiled sections were then exposed in a moist space, under otherwise parallel conditions: after the lapse of 24 hours the following observations were made:—

Boiled	Unboiled	Boiled	Unboiled
No visible color change	Intense coloration	No visible color change	Very intense blackening

The phenomenon is probably due to the action of the oxidising enzyme tyrosinase on the protein dissociation product tyrosin. The occurrence of tyrosin in potatoes has been definitely established by Studel[1], and by Schulze and Winterstein[2].

The more intense darkening invariably observed in series B and similarly treated material is probably due to the partial disorganisation and death of the cellular units, and the consequent freer diffusion of either the enzyme or tyrosin or both, from cell to cell.

Blight-free Intact Tubers. Sulphuric Acid Steeps.

The outstanding fact furnished by the foregoing experiments, viz., that even on prolonged steeping in brines ranging from 10 per cent. to 20 per cent. strength, the dissolved substance only entered the steeped unpeeled tuber in small amount, led to the institution of experiments with aqueous solutions of sulphuric acid. In each of the experiments summarised in the tables beneath, five tubers were selected and separately steeped in a 5 per cent. or 10 per cent. solution of sulphuric acid, for the time periods indicated, and at laboratory temperature, 16° - 18°Co.

At the termination of each experiment the material was removed, washed, divided into two lots of three and two tubers

1 Deutsche med. Woch. 1900, p. 273.

2 Zeitschr. f. physiol. Chem. 35, 299, (1902) and 45, 79, (1905).

each, the former planted in good garden soil (vitality test) and the latter stored in paper bags (storage test).

The immediate objects of these experiments were :—

(1) To establish as definitely as possible whether or not the vitality of the buds, and hence the sprouting power, was destroyed ; and

(2) To test whether or not the storage qualities of the different types of tubers were impaired, under the selected conditions of treatment.

Table 1.

Results of planting and storage of acid-treated tubers.

A.—Mature Victorian thick-skins.

5 per cent sulphuric acid.

Expt.	Date of treatment	Duration of steeping	Date of planting	Behaviour on (a) planting	Behaviour on (b) storage	Keeping quality of stored tubers
1	10/7/11	2 hours	10/7/11	3 growing	2 sprouted	good
2	10/7/11	6 ,,	10/7/11	0 ,,	2 ,,	,,
3	12/7/11	3 ,,	12/7/11	2 ,,	2 ,,	,,
4	12/7/11	1 ,,	12/7/11	3 ,,	2 ,,	,,
			10% sulphuric acid.			
5	13/7/11	5 hours	20/7/11	1 growing	0 sprouted	good
6	13/7/11	1 ,,	20/7/11	3 ,,	2 ,,	,,
7	19/7/11	20 ,,	20/7/11	0 ,,	0 ,,	,,
7a	17/7/11	3 ,,	20/7/11	0 ,,	0 ,,	,,
7b	17/7/11	2 ,,	20/7/11	0 ,,	1 ,,	,,

B.—Mature Victorian thin red-skins.

8	19/7/11	4½ hours	20/7/11	1 growing	0 sprouted	good
9	20/7/11	2½ ,,	20/7/11	1 ,,	1 ,,	,,

C.—Mature South Australian thin-skins.

10	18/7/11	3 hours	20/7/11	0 growing	2 sprouted	good
11	18/7/11	5 ,,	20/7/11	1 ,,	0 ,,	,,

These observations were made on August 24, 1911, and again on August 28, 1911 ; on the latter date the accompanying photographs were taken of the planting and storage test experiments.

Immersion for from three to six hours in either 5 per cent. or 10 per cent. sulphuric acid induces marked retardation of growth. In certain cases (Experiments 2, 7, 7a, 7b, 10) actual suppression of the sprouting powers of the tubers resulted. These results, although variable, are not surprising, when the variability of the material, and the possible individuality possessed by such biological material, are given due consideration.

The effect of the 10 per cent. solution is in no way doubtful; a steep in this concentration of acid, of 20 hours' duration, effectively and conclusively destroys the vitality of the buds of the treated tubers.

In addition to the numerical data in the above tables the accompanying Plates LVII., LVIII., LIX. (planting and storage experments) indicate both the retardation and the destructive influence exercised by sulphuric acid when used as a steeping reagent. The storage quality of these tubers was in every instance unimpaired. In the above experiments, after two months' storage, it was impossible to differentiate them from the untreated but otherwise similar tubers, which served as a control in these storage tests.

In experiments 7, 8 and 11, the amount of acid in definite volumes (100 c.c.) of solution was determined at the commencement and termination of the experiments; these results are given below:—

Expt.	Grams of acid per 100 c.c. of solution		Decrease in amounts of acid per 100 cc. of solution.
	At commencement of steeping	At termination of steeping	
7	9.408	9.359	0.049 grams.
8	9.408	9.408	0.00 grams.
9	9.506	9.408	0.098 grams.

It is evident that the amounts of acid which diffuse from the steeping medium into the tubers are relatively minute.

Since in each of these experiments 1.500 c.c. of acid solution were used, and the average weight of (1) 5 tubers was approximately 1.650 grams, all the necessary data are available for calculating the apparent percentage weight of acid absorbed.

Taking for example experiment 9, we have weight of acid withdrawn from 1.500 c.c. $= 15 \times 0.098$ grams.

Percentage of acid apparently present in tubers

$$\frac{15 \times 0.098 \times 100}{1650} = 0.089 \text{ per cent.}$$

As we shall shortly see, the distribution of the acid absorbed is not uniform, but localised in the buds and the immediately subjacent tissue.

Again it is probably more correct to state that the evaluation of the percentage of acid absorbed is too high, for the fall in the titre of the solution is partly accounted for by the adherence of the acid to the skin of the treated tuber.

So far no definite evidence has been adduced as to the localisation of the points of entry of the dissolved substances into steeped tubers.

As we have seen the amounts of sodium chloride and sulphuric acid which diffuse into the steeped tuber are relatively small, and while ordinary quantitive chemical determinations indicate with tolerable precision the general trend of the diffusion processes, these suggest rather than definitely establish the fact that absorption of the solute is exclusively confined to the buds.

The entry of sulphuric acid into the mature blight-free and undamaged tubers, it may be stated, takes place exclusively through the buds.

The point is capable of precise demonstration: the freshly-cut tuber, whether untreated or after steeping in water, on testing with an aqueous solution of methyl orange, gives a distinctly alkaline reaction. It follows, therefore, if, after steeping tubers in an aqueous solution of H_2SO_4 deeply coloured with methyl orange, for five or more hours, and then carefully washing with distilled water to remove the last traces of acid adherent to the surface, a persistent pink colour is demonstrable at any point beneath the skin, that both acid and indicator have been absorbed. If, for example, an intact tuber, the skin of which is undamaged, is thus treated, and sectioned so that the plane of section includes a bud or "eye," it will be found that the bud and the immediately subjacent tissue for the depth of a few millimetres, is more or less distinctly mapped out by a pink coloration, which is intense superficially, and becomes gradually fainter as it is traced internally. That methyl orange

alone does not give this colour reaction may easily be controlled by steeping similar tubers in an aqueous solution of the indicator containing approximately the same concentration of methyl orange as the acid solution.

These facts therefore justify the conclusion that the entry by diffusion of the dissolved substance so far tried is exclusively *via* the buds or "eyes" of intact mature tubers.

The point demonstrated is, as we shall shortly see, of importance when we come to deal with the behaviour of blight-infected tubers on exposure to the action of aqueous sulphuric acid.

Plate LX. shows the appearances presented by such a tuber as that described above, after 20 hours' steeping in 10 per cent. aqueous sulphuric acid plus methyl orange.

Blight-free Intact Tubers. Boracic Acid Steeps.

The steeping of intact tubers in solutions of common salt and sulphuric acid, as we have seen, is attended by very restricted diffusion of these reagents through the skins of the steeped tubers. It was of interest to investigate in this particular respect the action of boracic acid.

This acid has a much lower solubility than either of the reagents mentioned, functions as a comparatively mild antispetic, and in aqueous solution possesses certain physico-chemical properties which differentiate it from both NaCl and H_2SO_4.

The following experiment may be taken as typical of the results furnished by steeping intact tubers in aqueous solutions containing 1.61 per cent. and 0.63 of the acid.

Experiment:—Two lots (5 each) thick-skinned Victorian tubers were separately steeped in (1) 1.61 per cent. and (2) 0.63 per cent. boracic acid solutions for 10 and 11 days respectively, and the titres of the solution determined at the commencement and termination of the steep experiments.

	Grams boracic acid per 100 cc. of solution.	
	(1)	(2)
At commencement	1.61	0.63
At termination	1.60	0.65

The results are, as regards the comparative non-diffusibility of the dissolved substances through the skin or rind of the tuber, quite comparable with those derived from otherwise parallel experiments save that NaCl or H_2SO_4 was displaced by boracic acid.

It may be definitely stated that boracic acid like NaCl and H_2SO_4 in aqueous solution, under the conditions of experiment indicated, does not diffuse through the skin of the tuber.

The usual planting, storage and cooking tests were carried out with this material; no signs of growth were apparent, even after a lapse of two months, thus showing that the treatment had effectively extinguished any residual vitality the buds of the treated tubers may have possessed prior to the institution of the planting tests. The storage and cooking tests were quite satisfactory, the treated tubers retaining their normal solidity, cooking well, and being quite wholesome.

A small parcel of tubers immersed in a 2 per cent. boracic acid solution, contained in tins which prior to shipment were hermetically sealed, were sent from Melbourne to Perth. These arrived in good order, little or no bacterial activity was evident in the solutions, and the tubers on opening the tins were found to be quite sound and solid. After exposure, however, parts of the surface of each tuber became much discoloured, and in these superficial areas bacterial decay set in and rapidly progressed.

It is highly probable that during transit the tubers were unavoidably bruised at the parts of their surfaces at which, after removal from the solution, discoloration and eventually decay of the subjacent tissue took place. As possible injury by bruising during the laboratory operations was both controllable and avoidable, the tubers in my experiments did not exhibit these degenerative changes.

It is interesting to note that in these experiments with boracic acid as the steep-medium little or no bacterial development was apparent.

The results afforded by the foregoing experimental data justify the following conclusions:—

(1) When mature blight-free tubers are steeped in aqueous solutions of either common salt, sulphuric or boracic acid, under

the conditions of time, temperature and concentration of salt or acid stated, only minute amounts of the dissolved substance diffuse into and are absorbed by the steeped tubers.

(2) If the skins of mature intact tubers are undamaged, the entry of sulphuric acid takes place exclusively via the buds or "eyes" of the tubers, and the same statement applies, at least during the earlier phases of the steep experiment, in reference to the entry of NaCl and H_3BO_3.

(3) If the steeping in sulphuric acid is restricted within certain limits of time and concentration of acid, the vitality of the buds or "eyes" of the tubers is unimpaired or not greatly impaired. If these limits are exceeded, the vitality of the buds is effectively destroyed, without, however, seriously damaging either the cooking and edible or storage qualities of the treated tubers provided that the period of storage is not inordinately prolonged, and dry and airy storage conditions are maintained.

Blight Infested Intact Tubers. Steeps in Aqueous Solutions of Various Reagents.

The stages of the enquiry so far related refer exclusively to the treatment of blight-free tubers, the entry, mode of entry, and action on the vitality of the buds of the treated tubers of certain chemical substances, under controllable conditions of experiment. The accumulation of convincing and definite evidence on these points, and the possible practical application of the knowledge acquired, rendered it desirable to extend the inquiry to the investigation of blight-infected material.

It became, for example, a matter of special interest to investigate the possible influence one or other of the methods of treatment essayed might exercise on the vitality of the hibernating mycelium of the blight-inducing fungus, which, it is very generally accepted, resides in the infected tuber; this latter being regarded as the chief vehicle of dissemination of this difficultly combatable disease from district to district, it may be from country to country.

The question may assume a form which is not devoid of economic importance. In certain circumstances it may be

desirable and justifiable for a State to close its ports and markets against the importation of tubers from another State, in which the crops furnishing the main source of seed-supply have, it may be very recently, been badly invaded by Irish Blight. On the one hand absolute prohibition of a particular foodstuff is a difficult and grave matter; on the other the introduction of disease-carrying seed tubers constitutes a grave menace to the local industry, especially if no previous and authentic history of its appearance exists, or if such has been authentically recorded, no recrudescence of the disease has manifested itself after the lapse of a period of time which may reasonably be supposed to have been ample for its complete disappearance.

It follows that if a means could be devised, at once not too costly or difficult of execution, whereby bright-infected tubers could be subjected to treatment which, while rendering them useless for seed purposes, would not impair their use for human consumption, then the dual menace suggested might effectively be surmounted.

The importation of blight-carrying tubers might then be permitted without risk of introducing or re-introducing diseased material, and moreover its subsequent dissemination by planting affected tubers might be practically if not completely eliminated.

As we have seen, although prolonged steeping of blight-free tubers in aqueous solutions of sulphuric acid under certain definite conditions results in (1) the complete destruction of the sprouting power of the tuber, and thus renders it useless for seed, it does not (2) seriously impair either its keeping or culinary qualities. The experimental work briefly described in the present section relates exclusively to the steeping of blight-infected material, of which various lots, differing in type of potato and degree of infection, were obtained and subjected to treatment on both a laboratory and a larger scale.

An illustrative example will suffice. Six lots (15-20 each) of tubers were separately steeped for ten days as under:—

Experiments 1, 2, 3, 4, 5, 6 in solutions indicated in the following table.

Experiments 7 and 8—unsteeped controls.

After four days' immersion, specimens (five tubers in each instance) were removed from experiments 1, 2, 3 and 6, and separately "forced"—i.e., placed under favourable conditions of temperature (65° to 70°F.), and humidity for the development of the causal fungus and its characteristic fructifications.

It is to be noted that in all these forcing tests the necessary safeguards against possible cross-infection were taken.

After ten days' immersion a second series of forcing tests was instituted, ten tubers from each experiment being separately forced, and subsequently investigated after expiration of 12, 24 and 28 hours. Included in this second series of tests were two untreated lots of tubers which served as controls. Of these one (a) was drawn from the same bulk as experiments 1-6, while (b) was derived from another source. The chief object aimed at in duplicating these control-forcing tests was to demonstrate beyond all reasonable doubt that the condition of experiment under which the "forcing" tests were carried out was such as to induce the active development of the hibernating mycelium which a previous trial of like material had also shown to be present.

The forced material was subjected to searching microscopical examination, and the results are briefly synopsised in the table beneath. Where obvious and undeniable evidence of the development of the blight fungus was demonstrable (experiments 7 and 8) is indicated by a plus (+) sign, where no sign of its presence was detected (experiments 1-6) a negative sign (−) signalises the fact.

Expt.	Treatment Material steeped in	Results of forcing tests 4 days		10 days
1	0.25 per cent. phenol solution	−	-	−
2	0.25 per cent. formalin solution	−	-	−
3	1.0 per cent. boracic acid ...	−	-	−
4	4.0 per cent. boracic acid ...	−	-	−
5	15 per cent. NaCl 1 per cent. bor. acid solution	−	-	−
6	Plain tap water	−	-	−
7	Control a	−	-	+
8	Control b	−	-	+

The evidence derived from these experiments indicates that when mature blight-infected tubers are steeped under the above conditions, examination of the material at four or ten days fails to reveal its presence. The evidence was just as conclusively negative in experiments 1-6 as it was positive in experiments 7 and 8, and it is difficult to interpret the results other than by stating that as the result of steeping infected tubers in both solutions (experiments 1-5), or in plain water (experiment 6) the hibernating mycelium of the fungus is completely suppressed. In the latter, it is not improbable, considering the marked aerobism of the fungi in general, that the effect is induced by asphyxiation through prolonged drowning.

Planting tests carried out with portions of the material from experiments 1 to 5, were entirely negative, while the material from experiment 6 underwent advanced decay, during the process of steeping.

Subsequent work in this direction was confined to steeping blight-infected material in aqueous solutions of 2.5, 5.0, or 10 per cent. strengths, for a uniform period of steeping of 16, and finally of 10 hours' duration. Different kinds of tubers varying considerably in degree and extent of infection, were used, and the magnitude of these latter experiments emerged from a laboratory to a practical scale, a sack ($1\frac{1}{2}$ cwt.) of tubers being steeped in each of these essays. The customary "forcing," storage and cooking tests were carried out in each of these experiments.

While a steep of 10 hours' duration in a 2.5 or 5.0 acid generally proved inadequate to suppress the vitality of buds and fungus, the results of steeping material for the same period in 10 per cent. acid were invariably negative, i.e., no development or growth of either buds or fungus ensued. The most careful microscopical examination failed to reveal the presence of the fungus; contemporaneous "forced" controls invariably gave positive results.

These results justify the conclusion that when blight-infected mature tubers are steeped in 10 per cent. aqueous sulphuric acid for 10 hours, the sprouting power of the steeped tuber and the hibernating mycelia of the blight fungus were completely destroyed.

Critical storage and cooking tests show that the tissue of these tubers undergoes only slight and tardy deterioration provided that these are only slightly infested with blight. Where extensive areas of the skin are involved, their storage quality is plainly defective and unsatisfactory. This statement equally applies even in the case of untreated tubers and particularly so under unfavourable conditions of storage.

It remained to subject blighted tubers to the test with 10 per cent. sulphuric plus methyl orange. Badly blighted specimens were steeped for varying periods—5, 10 and 20 hours, and sections of these tubers were prepared similarly to those earlier described. These when submitted to both macroscopical and microscopical examination, showed that wherever the skin was overlying tissue which had been invaded by the blight fungus, there the acid and indicator had freely entered and diffused into the subjacent tissue. Entry of the acid, therefore, is not exclusively confined (as in blight-free tubers) to the buds. In other words, while the undamaged healthy skin of the disease-free tuber functions as a semi-permeable or selective covering to the storage tissue beneath it, the damaged skin of the blight-infected tuber no longer possesses this property. The entry of the acid through the skin and buds is well shown in Plate LX.; in the original objects which these plates represent, the lighter diffusion zones or areas are very clearly differentiated from those portions of the tissue into which the acid has not diffused, by a pink coloration (1).

Certain observations by Wütrich[2] on the toxicity of H_2SO_4 in dilute concentrations on the conidia and zoospores of *Phytophthora* are of interest.

In each instance the period of observation covered 15 hours, and the following results are recorded:—

Strength of acid	*Phytophthora infestans*, De Bary.
0.0049 per cent - - -	Within 15 hours conidia neither form swarm spores nor directly germinate

1 Sections should be examined as early as possible after cutting, since after a few hours, desiccation at the surface leads to the diffusion of alkali.

2 Zeit. f. Pfl. 1892, 16-31, 81-94.

Strength of acid	*Phytophthora infestans*, De Bary.
0.0049 per cent and malt extract	Conidia, an injurious effect is evident
0.0098 per cent and malt extract	Conidia, absolutely no germinative processes take place
0.0049 per cent - - -	Zoospores, motility of spores immediately arrested, germination suppressed.

The amount of acid which diffuses into the treated undamaged tuber, calculated as a percentage on total weight of tissue is 0.089 per cent. In the case of blight-infected material, the percentage absorbed is certainly higher. In either case, the diffused acid is distinctly localised and consequently in those portions of tissue into which it has penetrated the percentage will be greater than the above value.

In view of Wütrich's observations, the death of the fungal mycelia after the treatment of blight-infested tubers, with 10 per cent. acid is therefore easily conceivable.

Summary.

The results embodied in this paper may be briefly summarised :—

(1) When intact, blight free, mature tubers are steeped in aqueous solutions of the various chemical substances enumerated in this paper, the entry of the solute during the earlier stages of immersion is chiefly if not solely *via* the buds of the tuber.

(2) A steep of 10 hours' duration in a 10 per cent. solution of aqueous sulphuric acid suffices to destroy the vitality of the buds of the tubers, steeped in this reagent.

(3) A steep of 10 hours' duration in a 10 per cent. solution aqueous sulphuric acid suffices to destroy the vitality of the buds and also that of the fungal mycelia in the case of blight infested tubers. The solute in this case not only enters *via* the buds but

also through those parts of the skin which have been damaged by the ravages of fungus, or other parasitic organisms.

(4) It is essential to add that the results so far related in regard to the storage qualities possessed by acid treated tubers refer solely to the results obtained with fully matured, sound tubers.

It is hoped that facilities will shortly be provided for the further prosecution of this investigation.

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EXPLANATION OF PLATES.

Plate LVII.

Mature Victorian thick-skinned tubers, 10 % H_2So_4 treatment; experiments 5, 6, 7, storage tests.

Plate LVIII.

Mature Victorian thick-skinned tubers, 10 % H_2So_4 treatment; experiments 5, 6, 7a, and untreated control planting tests.

Plate LIX.

Mature Victorian thick-skinned tubers, 10 % H_2So_4 treatment, experimenting 7, planting test. Three tubers on removal from soil 40 days after planting.

Plate LX.

Entry of acid into blight-free and blight-infested tubers.

Expt. 1.—Cross section of blight-free tuber after steeping 20 hours in aqueous methyl orange. The arrows in

this and succeeding figures indicate the position of the bud.

Expt. 2.—Cross section of blight-free tuber after steeping 20 hours in 10 % H_2So_4 and methyl orange. It will be noted that the entry of the acid is exclusively confined to the buds.

Expt. 3, 4, 5.—Cross section of blight infested tubers after 20 hours steeping in 10 % H_2So_4 and methyl orange. The points of entry of the acid through portions of the skin damaged by the ravages of the blight fungus are shown.